

The Evolution and Determination of Sexual Characters in the Angiosperm Sporophyte*

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One result of genetic study which bears definitely upon evolutionary theory is the demonstration that the determination of an apparently simple character depends upon the activity of many genes. It is indeed suggested that the interaction of all the genes of an organism may be essential to the appearance of any character; but for the present this broader conception remains in the realm of speculation.

Another contribution from the same source is the demonstration that similar or apparently identical phenotypes may be determined by diverse genic complexes. It follows that very different gene mutations in distinct lines of descent may result in the appearance of similar characters—a fact which in another aspect students of phylogeny have long stated in terms of parallel or convergent evolution.

Turning to a special class of characters, it is evident that sexual differentiation has arisen independently in many different plant and animal lines. There is no reason for assuming that the changes in the genetic mechanism which resulted in this differentiation were identical, or even closely similar, in diverse lines.

To this consideration is to be added that, after the first step in sexual differentiation, additional mutations occurred, independent and varying in different lines. These later steps resulted in differentiation between the organs in which gametes are produced; in differentiation of individual gamete-producing plants or animals as respectively female and male; and, in certain pteridophytes and in all seed plants, in a backward extension of sexual differentiation to involve structures of the parental spore-bearing generation.

A priori, then, it is not to be expected that the genetic mechanisms which determine sexual potentialities or which influence sex-expression should be the same in different groups of organisms. Yet it is characteristic of discussions in this field that unitary theories of "sex-determination" have been developed; each based upon phenomena observed in one or in a few related species, but each seeking to apply one mechanism to all groups of sexually differentiated organisms. There is, to be sure, one set of facts which may seem to support this conception of uniformity: namely, the occurrence in widely separated phyla of apparently similar bodies—the sex chromosomes—which are a part of the genetic mechanism whose nature is being sought.

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But it would not be surprising to discover that this type of similarity presents an additional instance of parallel evolution.

The history of angiosperms begins at a level at which the greatly reduced members of the gamete-bearing generation had long been sharply separated as female and male individuals. Sexual differentiation also had been projected backward to effect a distinction in the parental generation between female and male spore-bearing structures. These structures—macro- and micro-sporangia—were now borne upon or within likewise sexually differentiated organs later to be designated pistils and stamens.

Less confident must be any statement as to the *distribution* of pistils and stamens in primitive angiosperms. Three conditions are conceivable. Either the original angiosperm flower was bisexual (bisporangiate), the plant bearing it being hermaphroditic; or there were separate pistillate and staminate flowers, borne either on the same plant (a condition of monoecism) or on distinct plants (a condition of dioecism).

Attempts to choose between these possibilities were based first upon comparative morphology; then, as fossil evidence accumulated, the assistance of paleobotany was sought. The latter source has as yet contributed little to the problem here involved. It has shown that the equivalent of a bisporangiate flower was developed by Cretaceous times in the Bennettiales; and that the equivalent of a unisporangiate flower was present in the Caytoniales as early as the Triassic. But it is agreed that neither Bennettiales nor Caytoniales were ancestral to modern angiosperms. Probably the great majority of those who have discussed the question have concluded that primitive angiosperms had bisexual flowers. But unanimity upon this point is not reached; and the possibility of a polyphyletic origin, some lines starting with hermaphroditism, others with monoecism or dioecism, is not wholly excluded.

Since the sharp distinction between female and male gametophytes was established at a pre-angiosperm level, a discussion of the evolution of sexual characters in angiosperms can deal only with developments within the spore-bearing generation. It may be asked, first, what if any genetic evidence is there as to the type of distribution of sexual structures in primitive angiosperms? Second, what appears to have been the most probable course or courses of evolution of sexual characters since the dawn of angiosperm history?

Two general sets of facts, long recognized and both to be referred to later, suggest the derivation of unisexual from bisexual flowers. One of these concerns the presence in the majority of monoecious and dioecious species of pistil-rudiments in staminate flowers and of stamen-rudiments in pistillate flowers. The stage to which these rudimentary structures develop varies from that of a small hump of undifferentiated tissue to that of the reflexed stamens of the functionally female flowers of the grape, which produce non-viable or

rarely viable pollen. The appearance of such variably developed but functionless or chiefly functionless organs is difficult to explain save by reduction in the course of descent from a hermaphroditic ancestor. A different conception may be based upon those species whose unisexual flowers show no trace of organs of the opposite sex. When, however, such plants belong to, or are obviously closely related to, families among whose members are some with bisexual flowers or with unisexual flowers containing staminodia or pistillodia, the obvious explanation of unisexuality is still that of descent by reduction from a bisexual condition. There remain the relatively few species that supply no indication, through either structure or relationship, of such descent. It was this condition in *Casuarina* which made it, in Wettstein's phylogenetic scheme (19), the starting-point for angiosperms.

The other set of facts with a similar bearing is the variability of sexual conditions in those angiosperms whose flowers are typically unisexual. In many monoecious and dioecious species, bisexual flowers now and then appear. Even more frequently, staminate replace pistillate flowers and *vice versa*; flowers of either sex appearing on the dioecious plant or on the part of the monoecious plant which regularly bears flowers of the opposite sex. Whatever its explanation, such lability of sex-expression in the sporophyte contrasts sharply with the rigid separation of sexual characters in the gametophyte. Comparable lability seems to characterize gyno- and andromonoecious, gyno- and androdioecious species.

In this connection, too, cases may be found which could be thought to point in an opposite direction. Among the dioecious species that have been extensively studied, three (two of *Lychnis* and one of *Bryonia*) present a very sharp sex-separation. Doubtless, when other less well-known species are studied, similar instances will be found. But in *Bryonia dioica* staminate flowers, in *Lychnis dioica* and *L. alba* both staminate and pistillate flowers, contain rudiments of organs of the opposite sex. The change in sex-expression in pistillate flowers or *Lychnis* under the influence of the anther smut is well known, although human ingenuity has yet found no means of accomplishing a like result. No modification of sex-expression is known to have been induced in *Bryonia dioica*, though there is one old report of a female plant bearing some bisexual flowers.

The common variability of the unisexual condition is among the facts which long ago led to the conclusion that in all angiosperms genotypic bases are present for both femaleness and maleness. No reason has appeared to question this conclusion; indeed, all later-adduced evidence has but served to confirm it. Correns (2) postulated for dioecious species an additional gene or gene-complex for sex tendencies superposed upon those representing sex potentialities. This formulation, recognizing a certain degree of genotypic

complexity, is still, so far as it goes, valid as a formal statement of the case. As will appear, it is now evident that the story is even more complicated than Correns' statement would imply.

A first suggestion of the complexity to be expected appears in the fact that in dioecious angiosperms as in metazoa there are two general types of genotypic influence upon sex. In one, the more common type in both groups, the male is heterozygous, the female homozygous, for sex-tendency factors as well as for sex chromosomes. In the other, represented by strawberries and possibly by members of a few other genera, the male is homozygous, the female heterozygous. The difference is explicable by descent from hermaphroditic ancestors, different mutations in which have led to opposite results. It adds to the improbability of an assumption of the primitiveness of dioecism.

A type of mutation observed in a considerable number of hermaphroditic species involves a stoppage at some stage in the development of stamens (or their complete failure to develop), with the result either that no pollen is produced or, if produced, it is nearly or quite functionless. "Male-sterile" mutations of this general nature have been studied, for example, in the sweet pea, shepherd's purse, sorghum, *Oenothera*, onion, tomato, potato, barley (16). In these and in other plants, the condition in question seems to be due to a mutated gene (or to at least two genes in the tomato), the mutation being always or nearly always recessive. Mutations of a somewhat different sort bring about a replacement of stamens by petals or petaloid structures. It is clear that mutations of both types have occurred on a large scale in the past; witness the frequent occurrence, previously noted, of staminodia or stamen rudiments replacing some of the stamens in bisexual flowers or all the stamens in flowers which are now unisexual. Notable are the partial petaloid transformation of the last-remaining stamen of *Canna*; the often-observed occurrence of doubleness in consequence of a transformation of stamens; and the evidence from the morphological side that petals in many cases represent transformed and sterilized stamens. Mutations tending toward male sterility occur likewise in monoecious species. In maize, the most studied genetically of all plants, at least 27 distinct mutations of this general nature have been observed (7); 20 classed as "male-sterile," 5 as "tassel ear," one each of "antherless" and "pollen lethal." These 27 mutations involve as many distinct gene loci; all but two are recessive.

Such mutations in the direction of male sterility might be described as tending toward femaleness. Those of another type, known for example in *Silene*, *Cheiranthus*, and *Papaver*, in which stamens are replaced by carpels, may be similarly classed.

Comparable with the mutations which result in or tend toward male sterility are those leading toward female sterility. The striking fact shown

by a review of the literature is that mutations of this type appear, in both hermaphroditic and monoecious species, to be far less frequent than are those leading toward male sterility. Female sterility, pistils being more or less aborted, seems to be recessive in mutants of *Phleum pratense*, *Antirrhinum*, rice, and raspberries. In *calycanthema* forms of *Campanula* and of *Rhododendron*, the mutant condition (pistils developed but sterile) is dominant. In *Geranium*, pistils functional as such but showing structural transitions toward the staminate character have appeared in interracial crosses. The behavior of the character in back-crosses, while not entirely clear, suggests a Mendelian segregation. In a cross between species of *Geum*, the results of further matings are likewise not clear-cut. It is possible that in these crosses, as apparently in a few interspecific crosses which have resulted in male sterility, cytoplasmic influences are involved.

In the same list of mutations in maize which shows 27 genes involved in male sterility, only 6 mutations leading toward female sterility are reported: two "anther ear," 2 "barren stalk," 1 "lethal ovule," 1 "silless."

To the story of observed mutations in this direction must be added the known cases of pistillodia, which represent the result of past mutations; possibly the reduction in number of ovules in certain lines; and the relatively few cases of doubleness which have involved the transformation of pistils as well as of stamens into petals.

To be mentioned also are a few known mutations which, like two observed in intervarietal crosses in *Oryza* (12), tend simultaneously toward both male and female sterility. Obviously mutations of this class can hardly have played a direct part in evolution. In general they seem to result in monstrosities which, even apart from the accompanying sterility, would probably not be favored by selection. Chromosomal changes may be involved.

It is not yet clear why mutations toward male sterility are much more frequent than those toward female sterility. This difference holds not only for observed mutations. As to the past, it is evident that petals in a large proportion of instances represent sterilized stamens; only rarely can they be considered sterilized carpels.

The mutations thus far cited involve changes in the general direction from hermaphroditism toward dioecism. As already mentioned, very many variations (to be distinguished from mutations) occur in the opposite direction—involving the appearance in monoecious or dioecious species of bisexual flowers or, in dioecious species, of both pistillate and staminate (and sometimes bisexual) flowers on the same plant. Such variations are in large measure shown to be reactions to environmental conditions. They may be considered expressions of genotypic possibilities present from a remote

ancestry, certain of which have been inhibited, but not completely suppressed, by mutations like those previously mentioned.

But apart from variability of this common type, now and then a demonstrable *mutation* occurs in the direction from dioecism or monoecism toward hermaphroditism. Such mutations are known in dioecious species of *Lychnis*, *Salix*, *Silene*, *Vitis*, and *Fragaria*; in *Salix* and *Silene* they have occurred in the offspring of interspecific crosses. One recessive mutation resulting in bisexual flowers is known in maize (7). Most monoecious or hermaphroditic strains derived from dioecious species and subjected to genetic experiment have behaved as though they were mutated males; a very few have seemed to be mutated females. In *Lychnis* both mutated males and mutated females have been recognized cytologically. Those hermaphrodites (the term is often somewhat loosely used) which appear to be mutated males in general behave in breeding like males; that is, their progeny shows them to be heterozygous for a sex-tendency gene. In this respect they differ from regularly hermaphroditic species, which of course transmit hermaphroditism uniformly to all their progeny. This genotypic difference, as Correns pointed out, justifies the description of the appearance of hermaphroditism in a dioecious or monoecious species as a case of "backward evolution." The implication is that in a dioecious species derived from a primitively hermaphroditic one a mutation has produced a reversion to the phenotypically original character—although this change is not due to a reverse mutation of a previously mutated gene.

Not always readily distinguishable from these variations and mutations are the reported cases, in species classed as dioecious, of strains which regularly vary in degree of sex-separation. In *Urtica cannabina*, *Spinacia*, and *Mercurialis*, for example, plants shown to be genetically distinct occur which are monoecious or hermaphroditic. In other instances differing degrees of sex-separation are manifested by different strains. Comparable but not fully elucidated cases are presented by gynodioecious species. In the absence of direct evidence as to their origin, these diverse conditions are capable of explanation either as steps in an evolutionary sequence leading toward dioecism, individuals showing intermediate conditions not yet having been eliminated; or as evidences of mutation in the reverse direction comparable with the cases studied in *Lychnis*.

Mutations, then, may and do occur both in the general direction from hermaphroditism toward dioecism and in that from dioecism toward hermaphroditism. Those of the latter class are much the less frequent, and the best known of them lead to a hermaphroditism which is not genetically like the hermaphroditism which may be considered primitive. It is evident, too, that mutations away from hermaphroditism have been numerous in the past

and that, as shown by the persistence of rudimentary structures, very many of them have become fixed as part of the specific genotype.

The conclusion indicated by genetic evidence hence agrees with that most strongly suggested by morphological study; the general tendency in angiosperm evolution has been from a primitive hermaphroditism toward dioecism. In many species various intermediate stages have been reached: in something less than 5000 according to available counts (20), the final step to dioecism has been taken.

The mutations that have been chiefly concerned in the evolution of sexual conditions in angiosperms have involved a diminution or loss of the power of spore-production; commonly also a loss or reduction of the organs concerned. The mutations of this nature which are appearing at present are with rare exceptions recessive. It is reasonable to assume that similar mutations in the past history of angiosperms have, at their origin, likewise been chiefly recessive. Those mutations which have played the major rôle in floral evolution agree, therefore, in two respects—in involving a loss or diminution of potentialities and in being originally recessive—with the general run of observed mutations in all organisms. So far, then, as concerns one important group of structures and functions within one subdivision of the plant kingdom, evolution has proceeded by means of the type of mutation which genetic study has shown to be the prevalent type. In connection with this particular phylogenetic problem, the familiar difficulty of reconciling "progressive evolution" with genetic results does not arise.

It may be added that the mutative changes here shown to have been important are in harmony with the tendency toward the sterilization of sporogenous tissue which has characterized the evolution of bryophytes, pteridophytes, and seed plants.

The succession of steps in the changes from primitive hermaphroditism must remain for the present speculative. Obviously male sterility and female sterility may appear in different plants of a single species, as has happened in *Rubus* (4). In *Rubus*, however, dioecism is not yet reached, for matings of certain male and certain female plants produce some hermaphrodites and some "neuters" (without functional stamens or pistils). The species may at present (not considering the neuters) be classed as trioecious. At least two additional genetic changes would seem to be necessary (11) in order for the ultimate goal to be reached. Since mutations are likely to occur independently, it is to be expected that in the transition from hermaphroditism species now dioecious have passed through several intermediate stages.

It is possible to imagine the early steps to have been by way of gynodioecism or andromonoecism, trimonoecism, or monoecism. Any of these conditions could conceivably be reached by the establishment in homozygous condition

of one or (more probably) more than one mutant gene. How dioecism may arise from monoecism is illustrated by the success of Jones (8, 9) and of Emerson (6) in the production of dioecious races of maize through the selection of appropriate mutations. In each case two mutations were involved; and in each of the three dioecious races obtained, one pair of chromosomes differed with respect to a mutant gene which is epistatic to the mutant allele of the other selected pair.

Another conceivable transition from hermaphroditism to dioecism is by way of gynodioecism, which has been considerably studied, or of androdioecism, about which nothing is known genetically. If within a hermaphroditic species male sterility becomes a fixed character of one strain, evidently other strains of the species must usually retain functional pistils if the species is to persist—that is, a condition of gynodioecism must ensue. An alternative would be the development (by an additional mutation) of a structurally female but functionally parthenogenetic species. This seems to have happened in *Hieracium excellens* (14); but such mutational coincidences must be rare. A mutation (or mutations) transforming the hermaphrodites of a gynodioecious species into males would lead to dioecism.

The difficulty of explaining the behavior of gynodioecious species by any simple genic scheme led Wettstein (18) to the assumption of a cytoplasmic influence—an idea tentatively accepted by Correns (3) and recently emphasized and generalized by Lewis (10, 11). Apart from the inadequacy of an explanation based upon one or two mutations, the argument for a cytoplasmic inhibition in the female upon the functioning of male-tending genes rests upon the demonstration of such an apparent influence in several typically hermaphroditic plants, including forms of *Linum*, *Nicotiana*, *Geranium*, *Epilobium*, and *Streptocarpus*. With the exception of one case in maize (15), the known phenomena of this nature are limited to interspecific hybrids. On the other hand, also, gene mutations leading to male sterility are, as has been seen, of frequent occurrence. It is entirely possible that, when the variable behavior of gynodioecious species becomes better known, a (perhaps complicated) Mendelian explanation may be found possible.

Nearly twenty years ago Emerson (5), pointing out that "there are at least nine pairs of genetic factors which influence the expression of sex in maize," suggested that "the genetic situation in maize . . . may perhaps afford some help toward a solution of sex problems." The prophecy has been abundantly confirmed. Today more than 40 genes are known in maize, borne on at least 9 of the 10 chromosomes, whose presence in the "normal" or usual condition is directly essential to the sex-expression typical of the species as it exists at present. There are others, likewise essential in this regard, whose more conspicuous influence is upon the form, size, or vigor

of the plant. There can be no serious doubt that the sex-expression of other angiosperms, many of whose mutations parallel those observed in maize, is likewise dependent upon the activity of many genes.

A large proportion of the genes concerned in the sex-expression of contemporary species may have come down unchanged or little changed from primitive angiosperms. Those early species were themselves the outcome of a long evolutionary history in whose course had been developed a complex genotype. As has been seen, the passage from hermaphroditism to other sexual conditions need involve only a comparatively small number of mutations of genes already present. One step—perhaps in general the final step if maize may serve as an example—involved the establishment in some members of heterozygosis with reference to one pair of genes as to which other members of the species are homozygous. The pair of chromosomes bearing this allelic pair now plays a part in sex-determination.

Again to judge from maize, the selection of different mutant genes may in different cases give rise to the same phenotypic result—namely, dioecism. This example shows, too, how different pairs of chromosomes may in different cases come to function in sex-determination, as the X-Y pair appears to function in some seventy-odd species of dioecious angiosperms. The relation of the sex chromosomes to the differentiating genes may vary from species to species. In *Rumex* (13), as in *Drosophila*, the Y chromosome plays no demonstrable part in sex-differentiation. In *Lychnis*, on the other hand (17), its rôle is a positive one. In *Fragaria*, as in one of Emerson's derived races, the "X-Y" pair characterizes the female of the species; in all other well-known cases in angiosperms, this pair is the property of the male. While a partial picture is thus presented of the functioning of a pair of chromosomes in sex-separation, no satisfactory explanation is yet available for the frequent visible differentiation between the members of this pair. At the same time, it is shown in more than forty investigated angiosperms that there is no necessary correlation between the final genic differentiation which in a dioecist influences sex and a perceptible difference in chromosome size or appearance.

The mutations that have determined the transition from hermaphroditism have not produced in most cases, if in any, an absolute fixity of sexual character. Instead, whatever the inhibiting tendencies of a particular mutation, it remains possible, under favoring conditions, for some or all of the old potentialities to be manifested; as when bisexual flowers appear on a monoecist or dioecist. No rigidity of sex-separation seems to have been reached by the angiosperm sporophyte such as characterizes the angiosperm gametophyte or the gametophyte of a dioecious bryophyte.

The conception which emerges of the genetic basis for sex can not be satisfactorily formulated in terms of so many genes for maleness and so

many for femaleness. The elements which constitute this mechanism at any particular period in the history of a species influence in various ways and in varying degrees the development and functioning of stamens and pistils; they influence also the numbers and arrangement of these organs, whether in the same or in separate flowers, as well as the time of appearance of the respective flowers and their positions on the plant.

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